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Mechanism of Toxicity of Nitro Compounds Used in the Chemotherapy of Trichomoniasis

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The mechanism of the trichomonicidal activity of metronidazole and other 5-nitroimidazoles appears to depend on the ferredoxin-mediated reduction of their nitro group, with generation of a reactive metabolite or metabolites which interact with DNA leading to a subsequent inhibition of nucleic acid and protein synthesis. Redox cycling of these compounds under aerobic conditions appears to be a detoxification reaction by inhibiting net reduction of the drugs, thereby inhibiting their uptake. On the other hand, redox cycling of nitrofurans or other compounds with more positive reduction potential results in formation of high steady-state concentrations of oxygen-derived metabolites that might be of toxicological significance. It seems likely that reduced metabolites of nitroimidazoles (perhaps through covalent binding to tissue macromolecules and/or thiols depletion) are also involved in the nitroimidazoles' toxic effects to animal tissues and in their mutagenic and carcinogenic action.

Introduction

Human trichomoniasis, a chronic disease of the urogenital tract, is the most widespread of the sexually transmitted diseases (*1*). This disease is estimated to occur in about 20% of the female population of the U.S. (*2*). In fact, the Centers for Disease Control estimate that there are three million new cases of trichomoniasis each year, thereby surpassing the incidence of syphilis, gonorrhea, and genital herpes combined (*3*). Trichomoniasis is caused by *Trichomonas vaginalis*, a protozoan parasite of the vagina or the male urethra. Clinical manifestations of the disease vary even among patients of the same sex, since different strains of *T. vaginalis* seem to have different pathogenic capabilities (*1*). Symptoms may even worsen and improve repeatedly in the same patient. Consequently, while trichomoniasis in women is usually characterized by a copious, foamy, yellowish-green discharge that may have a foul odor, as well as mild to severe vaginal itching and burning, 25% of women harboring trichomonads have no symptoms at all (*1,4*). Although early studies associated trichomoniasis with cervical cancer (*5*), later studies have failed to confirm such a link (*6*). However, the prevalence of trichomoniasis is higher in women with cervical cancer than in healthy women (*7*). While trichomoniasis in men is most often asymptomatic, it may sometimes cause a slight urethral discharge which may or may not be accompanied by irritation, and in some cases it causes urethritis, prostatitis, epididymitis, and constriction of the urethra (*7*).

Trichomonas vaginalis is usually transmitted by sexual intercourse. However, it is generally acknowledged that in unusual circumstances the disease may be contracted through nonvenereal means (*7*), for example, from communal bathing water, contact with contaminated bath or toilet articles, and possibly even contact with urine on toilet seats (*8*). Infected women may also transmit trichomoniasis to their infant children during childbirth (*7*), and it has been suggested (*9*) that *T. vaginalis* may cause neonatal pneumonia.

Although other species appear to be potential pathogens, the ones of major importance in animal pathology are *Tritrichomonas foetus* in cattle and *Trichomonas gallinae* and *Trichomonas gallinarum* in birds (*10*).

T. foetus is transmitted as a true venereal infection in cattle. The disease may rarely be spread in other ways (*10*). The affected cow may show some evidence of vaginitis shortly after infection occurs, but usually this is overlooked. The affliction begins in the vagina and soon invades the uterus. As a result of this, the animal may fail to conceive. If conception occurs, the animal may abort within 2 to 4 months as a result of the infection. In other instances the fetus dies but is not discharged, in which case it becomes macerated and lies

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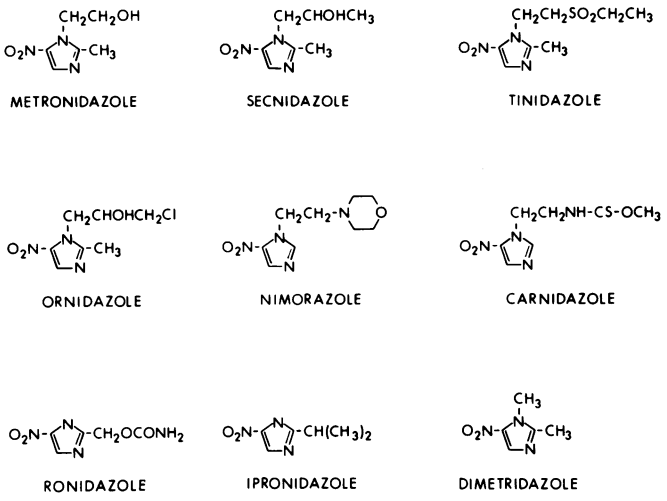
in a thin, nearly odorless fluid in which many trichomonads may often be found (*10*). Some authors have reported abortions of pregnant guinea pigs and rabbits by injection of *T. foetus* into the uterus (*11*). Artificial insemination and chemotherapy have reduced the importance of this disease in the U.S. but it is still of importance in other countries.

T. gallinae cause a disease affecting the upper digestive tract of pigeons, turkeys, chickens, and other birds (*10*), while *T. gallinarum* produces cecal and liver lesions in turkeys and chickens (*10*). Both these diseases are widely distributed and can cause severe losses.

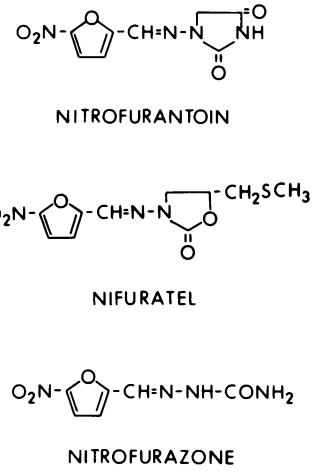
Since the early 1960s different 5-nitroimidazoles were introduced for the treatment of trichomoniasis. Metronidazole is the only member of the group available for human use in the U.S. (*1*). Dimetridazole, ipronidazole, and ronidazole are used to prevent infections in livestock (*12*), and tinidazole, nimorazole, carnidazole, ornidazole, and secnidazole are used in other countries as alternatives to metronidazole in the treatment of human trichomoniasis (*12–14*). In addition, several nitrofurans have been used for the treatment of human and animal trichomoniasis (*13,15–17*).



This review will focus only on the mechanism of toxicity of the nitro compounds currently used for the treatment of human and animal trichomoniasis. The literature on several nitroimidazoles and nitrofurans which have been used against trichomoniasis has been ably reviewed over the years by several authors (*18–29*). We have tried to minimize overlap with earlier reviews and put the emphasis on the most recent work.

Drugs Used in the Chemotherapy of Trichomoniasis

Until the late-1950s there was no successful, specific treatment for trichomoniasis (*30*). Nearly 150 different substances were then being used and recommended for human trichomoniasis, although none were particularly effective (*1,30*). The antitrichomonad activity of metronidazole [(1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole)] was reported in 1959 (*31*), and it was quickly recognized that this compound is active against a wide variety of anaerobic eukaryotic and prokaryotic microorganisms (*20*). Clinical use of metronidazole in the treatment of human trichomoniasis began in 1960 in Europe and in 1963 in the U.S. (*32*). During the more than 20 years since the discovery of metronidazole, a number of other useful nitroimidazoles have emerged. This group of compounds, which includes tinidazole, ornidazole, nimorazole, carnidazole, and secnidazole, is also clinically available for the treatment of trichomoniasis in different countries but shows no sufficiently improved activity over metronidazole for this disease (*19*). Certain nitrofurans such as nifuratel, nitrofurazone, and nitrofurantoin, active both *in vitro* and *in vivo* against trichomonads, have also been used in the treatment of human and cattle trichomoniasis (*13,15–17,33*), although its use in human trichomoniasis has been discontinued.



There is no doubt that nitroimidazoles are effective in combating trichomoniasis. Thus, cure rates of 95% or better can be expected from regimes of 200 mg of metronidazole given orally three times a day for seven days, or after a single oral dose of 2 g in humans (*34*). Nitroimidazoles are also effective against other diseases caused by other species of protozoans, such as amebiasis (caused by *Entamoeba histolytica*), and giardiasis (caused by *Giardia lamblia*). In addition, they are also effective against various anaerobic infections caused by a variety of anaerobic bacteria such as *Bacteroides fragilis*, Clostridia, Peptoestreptococci, Fusobacteria, and many others (*34*). They are commonly used to reduce the risks of infection by anaerobic organisms after colonic surgery (*34*). Finally, the ability of several nitroimidazoles to enhance the response of hypoxic cells to radiation (*35,36*) or cytotoxic agents (*37*) has attracted considerable interest in recent years.

Free-Radical Intermediates in the Toxicity of Nitro Compounds

The antiparasitic and genotoxic action of nitro compounds is generally agreed to require the enzymatic reduction of the nitro group, yielding a reactive metabolite. The complete reduction of the nitro group to an amino group requires six electrons per molecule. Theoretically, the reduction can occur in one-electron steps. The complete reduction scheme, including both diamagnetic and free radical intermediates, is shown in Eq. (1):

$$\underset{\text{(Nitro)}}{R-NO_2} \xrightarrow{e^-} \underset{\text{(Nitro anion radical)}}{R-\dot{N}O_2^-} \xrightarrow[2H^+]{e^-} \underset{\text{(Nitroso)}}{R-NO} \xrightarrow[H^+]{e^-} \underset{\text{(Hydronitroxide radical)}}{R-\overset{H}{\underset{|}{N}}O\cdot} \xrightarrow[H^+]{e^-}$$

$$\underset{\text{(Hydroxylamine)}}{R-NHOH} \xrightarrow[2H^+]{e^-} \underset{\text{(Amino cation radical)}}{R-\dot{N}H_2^+} \xrightarrow{e^-} \underset{\text{(Amine)}}{R-NH_2} \qquad (1)$$

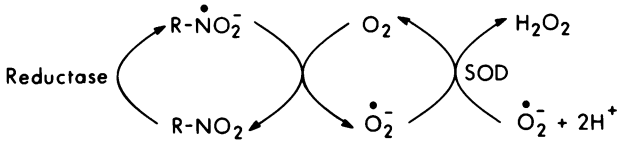
There are three potential free-radical intermediates of enzymatic nitroreduction: the nitro anion ($R-\dot{N}O_2^-$), the hydronitroxide ($R-HNO\cdot$), and the amino cation free radical ($R-\dot{N}H_2^+$). Electron spin resonance studies have demonstrated the formation of the nitro anion free radical intermediate in the reduction of nitro compounds by the oxygen-sensitive nitroreductases, which do not form diamagnetic products in the presence of air (*26*). No evidence is available so far on the formation of the hydronitroxide and the amino cation free radicals in nitroreductase incubations (*26*). Three chemical reactions of nitro free radicals could be of importance in the toxicity of these compounds (*26*). The first is the reaction of the free radical with itself, i.e., disproportionation:

$$2H^+ + R-\dot{N}O_2^- + R-\dot{N}O_2^- \rightarrow R-NO + R-NO_2 + H_2O \qquad (2)$$

This reaction might be either a detoxification reaction, because the free radical destroys itself, or the pathway to a more toxic species, such as the nitroso derivative (*26*).

A second, possibly important reaction is the covalent binding of the free radical metabolite to tissue macromolecules. However, recent experiments have demonstrated the unreactivity to DNA, proteins, or thiols of the nitro anion radical derived from several nitro compounds (*26,38*).

The third reaction, air oxidation of nitro anion free radicals is the dominant pathway in the presence of O_2 (*26,39*). This reaction results in regeneration of the nitro compound and production of $\dot{O}_2^-$ (*39*), whose dismutation yields H_2O_2 (*40*):



(3)

Again, this reaction might be either a detoxification reaction, because the free radical is oxidized and there is no net reduction of the nitro compound, or the pathway to more toxic species, such as oxygen-reduction byproducts ($\dot{O}_2^-$, H_2O_2, $OH\cdot$).

An alternative route for the radical anion has recently been proposed based on results obtained after UV and gamma-irradiation (*41*), and electrolysis (*41,42*) of metronidazole in aqueous media:

$$R-N\dot{O}_2^- \rightarrow R\cdot + NO_2^- \qquad (4)$$

This reaction involves the formation of nitrite ion (NO_2^-), and of a carbon-centered free radical ($R\cdot$). Although nitrite ion has been detected in these incubations (*41,42*), no evidence is available so far on the formation of a carbon-centered free radical upon nitroreduction.

Whether the radical intermediates, the diamagnetic products of nitroreduction, or the oxygen-reduction byproducts generated by the autoxidation of the nitro anion radical are involved in the antiparasitic and genotoxic action of nitro compounds will depend on several factors, i.e., nitro compound, presence of appropriate nitroreductases, presence of defense mechanisms against the oxygen–reduction products, aerobic or anaerobic habitat of the cell, etc. The work of the past few years has provided considerable insight into the mechanisms by which some of these nitro compounds exert their actions.

Mechanism of the Trichomonicidal Action of Metronidazole and Other Nitro Compounds

The anaerobic incubation of metronidazole with intact *Tritrichomonas foetus* (*43*) or *Trichomonas vaginalis* (*44*) cells in the presence of glucose determines the appearance of a characteristic ESR spectrum corresponding to the nitro anion radical. The signal is also observed in the absence of added glucose, indicating that endogenous reducing substrates can be used by the cells for metronidazole reduction (*43*). However, the steady-state concentration of the nitro anion radical is 40–50% higher with added glucose. Pyruvate is the most effective exogenous substrate for metronidazole reduction in incubations with *T. foetus* intact cells or homogenates. In addition, reduced pyridine nucleotides can also be used for metronidazole anion radical formation by *T. foetus* homogenates (*43*).

The ability of intact *T. foetus* to reduce nitro compounds is not limited to metronidazole. Incubation of several nitrofurans (nifuroxime, nifurtimox, nitrofurantoin) (*45*), 2-nitroimidazoles (benznidazole, misonidazole) (*43*), and 5-nitroimidazoles [ronidazole, secnidazole (*43*), MK-436, fexinidazole (unpublished results)] with intact *T. foetus* also generates multi-line ESR spectra corresponding to their respective nitro anion radicals.

The effectiveness of pyruvate as electron donor for metronidazole reduction indicates the participation of the pyruvate:ferredoxin oxidoreductase-catalyzed reaction in this process. This reaction involves an oxidative decarboxylation of pyruvate in which the acceptor for the reducing equivalents is a ferredoxin (*46*). This ferredoxin is the natural electron carrier linking pyruvate oxidation [reaction (5) catalyzed by the pyru-

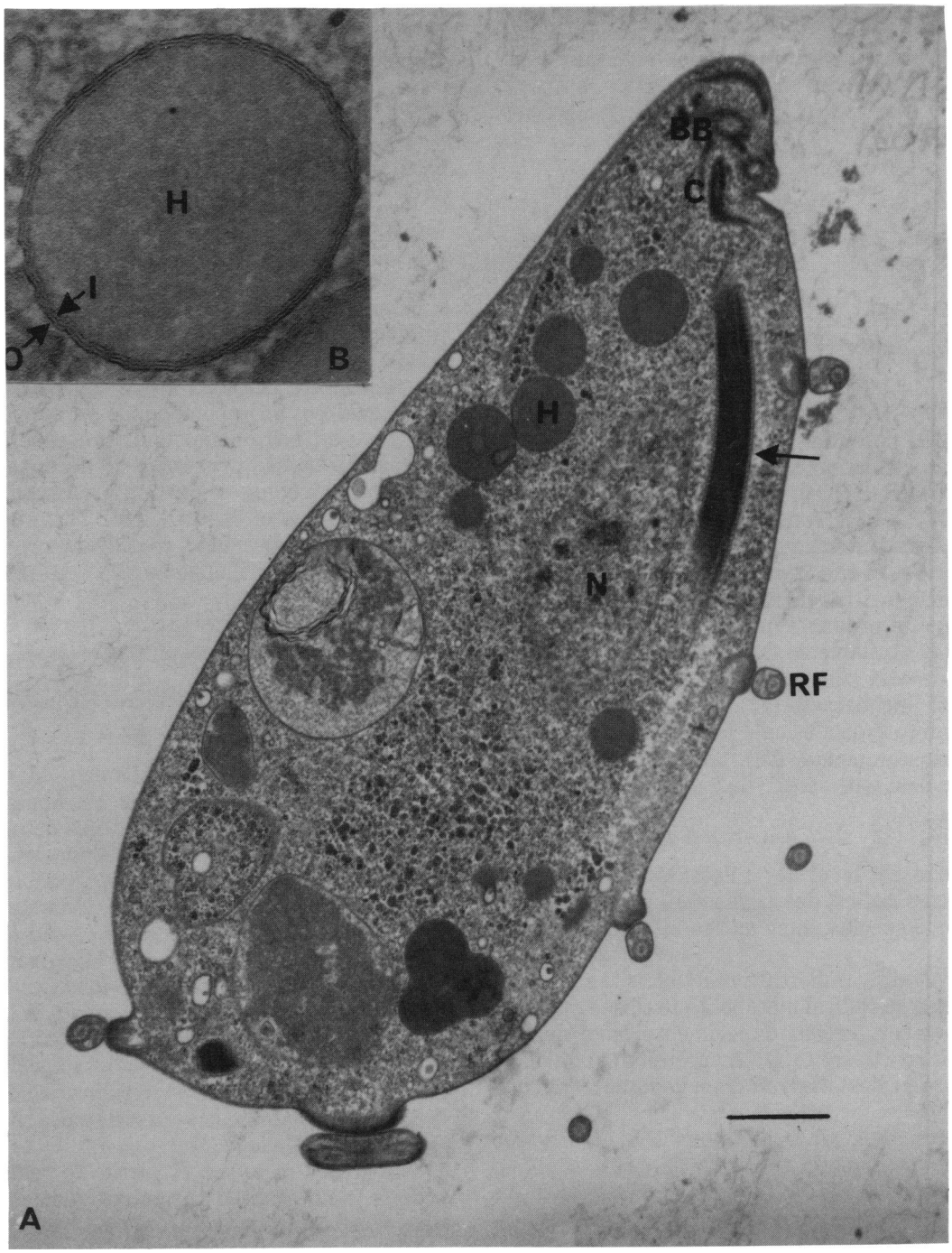


FIGURE 1. Electron micrographs of *Tritrichomonas foetus*: (*A*) Ultrathin section stained with uranyl acetate and lead citrate: BB, basal body; C, costa; H, hydrogenosome; N, nucleus; RF, recurrent flagellum. The arrow indicates the lateral arcuate system which surrounds the costa. × 14,000. Bar = 1 μm. From Benchimol et al. (*47*) with permission. (*B*) Cell fixed according to the glutaraldehyde-osmium tetroxide-potassium ferrocyanide procedure (*48*). The two closely apposed outer (O) and inner (I) unit membranes of the hydrogenosome (H) are clearly seen. × 66,000. Courtesy of Dr. Wanderley De Souza.

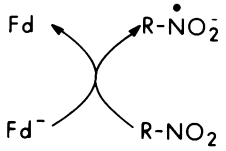
vate:ferredoxin oxidoreductase] to H_2 formation [reaction (6), catalyzed by the hydrogenase]:

$$\text{Pyruvate} + \text{CoA} + 2\,\text{Fd} \rightarrow \text{acetyl-CoA} + CO_2 + 2\,\text{Fd}^- + 2H^+ \quad (5)$$

$$2\,\text{Fd}^- + 2H^+ \rightarrow 2\,\text{Fd} + H_2 \quad (6)$$

Both these reactions occur in trichomonad hydrogenosomes, microbody-like organelles typical of these organisms (*46*) which are devoid of other typical eukariotic organelles such as mitochondria or peroxisomes (Fig. 1). Accordingly, the anaerobic incubation of metronidazole with the *T. foetus* hydrogenosomal fraction in the presence of pyruvate and CoA generates the metronidazole anion radical (Fig. 2) (*45*). The addition of purified ferredoxins causes a marked stimulation of the reduction of metronidazole to its anion radical (*45*), suggesting a role of a ferredoxin in this process. A stimulatory effect of ferredoxins on metronidazole reduction was first demonstrated in crude extracts of *Clostridium acetobutylicum* (*49*), *C. pasteurianum* (*50*), and *T. foetus* (*51*) supplied with pyruvate as electron donor. This stimulatory effect can also be observed in hydrogenosomal preparations from *T. foetus* or *T. vaginalis* supplemented with the purified ferrodoxin from the same organism (*52–54*). Since reduction of nitroimidazoles by ferredoxin-depleted hydrogenosomal extracts of *T.vaginalis* is still possible (*54*), it has been suggested that reduction of these compounds in the presence of ferredoxin is the sum of two processes, i.e., direct reduction by pyruvate:ferredoxin oxidoreductase and reduction mediated by ferredoxin. Accordingly, although the reduction rate of several nitroimidazoles tested depended on the one-electron mid-point potential (E_7^1) of the compound, the rate of additional reduction (stimulated rate minus basal rate) of all compounds was independent of the E_7^1 of the compound (*54*).

It has been postulated that trichomonad ferredoxins can be the acceptors of electrons from pyruvate through the pyruvate:ferredoxin oxidoreductase and that reduced ferredoxins can, then, reduce the nitroimidazoles (*20*) [Eq. (7)].



(7)

This reaction competes with the reaction catalyzed by the hydrogenase [reaction (6)]. Accordingly, trichomonads do not produce H_2 in the presence of metronidazole (*55*), and H_2 production resumes after all the added drug is reduced (*55,56*).

It is interesting to note that the metronidazole anion radical can also be observed in incubations of *T. foetus* hydrogenosomal fraction and NADH, although no stim-

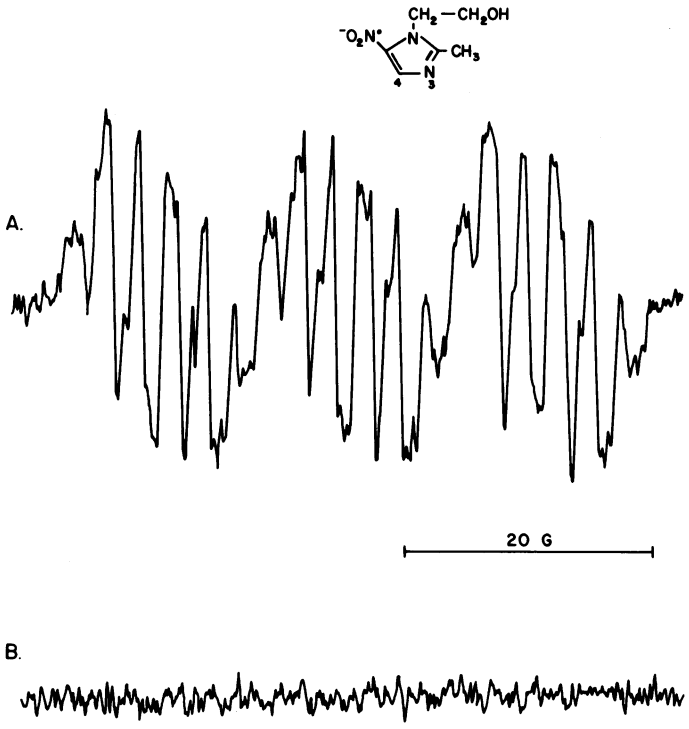


FIGURE 2. ESR spectra of *T. foetus* hydrogenosomes (*A*) in the presence and (*B*) absence of metronidazole: (*A*) The ESR spectrum of metronidazole anion radical observed after incubation of 10 mM metronidazole with 5 mM pyruvate, 1 mM CoA and *T. foetus* hydrogenosomal fraction (1 mg/ml) in buffer (pH 7.4). (*B*) Identical with (*A*) but incubation without metronidazole. From Moreno et al. (*45*) with permission.

ulation by ferredoxins is observed in this case (*45*). These results imply that pyruvate:ferredoxin oxidoreductase is not the sole system in trichomonads capable of reducing metronidazole (*45*).

In contrast with the results obtained with metronidazole, no stimulation of nitrofuran reduction is observed on addition of ferredoxins to *T. foetus* hydrogenosomes in the presence of either pyruvate or NADH as electron donor (*45*).

According to the proposed redox cycling of nitro compounds [reaction (3)], aerobic incubations of *T. foetus* hydrogenosomal fraction with metronidazole result in the consumption of O_2 and the generation of $\dot{O}_2^-$ and H_2O_2 (*45*). The addition of purified ferredoxins also causes a marked stimulation of metronidazole-induced O_2 consumption by these preparations (*45*). A very high concentration of metronidazole is necessary for the detection of an increase in the O_2 consumption and $\dot{O}_2^-$ and H_2O_2 formation by the hydrogenosomal fraction. In addition, metronidazole can not stimulate O_2 consumption in intact cells when used at low concentrations (less than 6 mM). Thus, redox cycling of metronidazole under aerobic conditions might be considered as a detoxification reaction by inhibiting net reduction of the drug, thereby inhibiting its uptake (*56*). The resulting low steady state concentrations of $\dot{O}_2^-$ and H_2O_2 are easily detoxified by the superoxide dismutase and catalase present in these parasites (*57–59*).

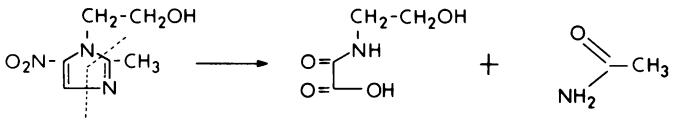
Recent experiments with *T. vaginalis* support the role of redox cycling of metronidazole as a detoxification reaction (*44*). Thus, a resistant strain has been postulated to have a greater rate of nitro anion radical disappearance attributed to a higher intracellular O_2 partial pressure due to a deficiency in its O_2-scavenging systems (*44*).

In contrast with the results obtained with metronidazole, lower concentrations of nitrofurans are necessary for the detection of an increase in the O_2 consumption and $\dot{O}_2^-$ and H_2O_2 formation by the hydrogenosomal fraction, and this process is not stimulated by ferredoxins (*45*). In addition, nitrofurans stimulate O_2 consumption in intact cells when used at low concentrations (less than 6 mM) (*45*). These results imply that oxygen-derived radicals can play a role in nitrofuran toxicity under aerobic conditions and can explain the efficacy of nitrofurans in topical treatment of trichomonad infections of the vagina (*60*), where high concentrations of the drugs can be achieved in not strictly anaerobic conditions. In this regard, it is interesting to note that low concentrations of H_2O_2 have been shown to be toxic against *T. foetus*, and H_2O_2 has been used for the treatment of *T. foetus* infection in bulls (*10*).

The reduction of metronidazole and further air oxidation of the anion radical explain the rather puzzling observation, that, although the reduction-dependent uptake of metronidazole (*56*) is markedly diminished by air, it is not entirely suppressed (*56*). Thus, the lower toxicity of metronidazole against trichomonads under aerobic conditions (*56*) can be explained by this detoxification reaction, which inhibits the formation of the unstable intermediate that interacts with DNA (*61*). This interaction with DNA is believed to be the mechanism of toxicity (*62*). Although the nitro anion radical is probably not the unstable intermediate which binds to DNA (*26*), it must be an obligatory intermediate in its formation. Whether one of the other two radicals (hydronitroxide and amino cation radical) or a diamagnetic product of nitroreduction (nitroso, hydroxylamine, amine) is involved in metronidazole toxicity remains to be elucidated.

In conclusion, redox cycling of metronidazole under aerobic conditions might be considered as a detoxification reaction by inhibiting net reduction of the drug, thereby inhibiting its uptake. The resulting low steady-state concentrations of $\dot{O}_2^-$ are easily detoxified by the superoxide dismutase present in these parasites. On the other hand, redox cycling of nitrofurans or other compounds with more positive reduction potential will result in formation of high steady-state concentrations of oxygen-derived metabolites that might be of toxicological significance.

Further metabolism of metronidazole in trichomonads involves ring opening with formation of acetamide and possible *N*-(2-hydroxyethyl)oxamic acid [Eq. (8)], although only acetamide was identified in *T. vaginalis* incubations (*63*). The amount of acetamide formed under anaerobic conditions appeared to be two- to fourfold greater than that formed aerobically (*63*). However, these products of metronidazole metabolism lack the activity of the parent compound (*20*). The absence of activity in the end products is regarded as strong evidence that one or several intermediates of the reductive process of the nitro group are responsible for the observed cytotoxicity (*20*).



METRONIDAZOLE N-(2-HYDROXYETHYL) OXAMIC ACID ACETAMIDE

(8)

Incubation of *T. foetus* with (^{14}C)-metronidazole results in binding of the label to subcellular proteins and possibly to other components such as DNA (*56*). Metronidazole also inhibits the uptake of ^{14}C-labeled thymine and uridine in *T. vaginalis* (*62*). Thus, an interaction with DNA of the reactive metabolite or metabolites of metronidazole and the subsequent inhibition of nucleic acids and protein synthesis is the most widely held explanation for its toxic action on Trichomonas (*20*).

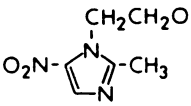
Free-Radical Metabolites of Metronidazole and Other Nitroimidazoles in Animal Tissues

Besides their wide use in the treatment of bacterial and parasitic disease (*20,64,65*), nitroimidazoles have been used in cancer therapy, both as radiosensitizers and as cytotoxic agents (*66–68*). Radiosensitization, hypoxic cell toxicity, and chronic aerobic toxicity correlate with the electron affinity (redox potential) of the nitroimidazoles (*68*). This similarity suggests that redox processes are involved in each phenomenon, but does not necessarily indicate a common mechanism (*68*). One of the major concerns in the clinical application of these drugs is that they may be cytotoxic to the normal hypoxic tissues in the human body (nerve, skin, cartilage, etc). For instance, side effects such as skin eruptions, polyneuropathy, and psychic disturbances are usually observed after prolonged treatment with nitroimidazoles (*34*).

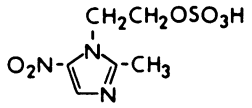
Metronidazole and ronidazole are reduced by rat liver microsomes to their nitro anion radicals, as indicated by ESR spectroscopy (*69*). They also increase O_2 consumption and $\dot{O}_2^-$ formation by rat liver microsomes. However, very high concentrations of nitroimidazoles are necessary to demonstrate these effects (*69*). These results imply that the effect of nitroimidazoles on $\dot{O}_2^-$ formation in mammalian tissues is small, hardly exceeding the basal levels. As occurs in trichomonads, redox cycling of nitroimidazoles under aerobic conditions might be considered as a detoxification reaction by inhibiting net reduction of the drugs. Since mammalian tissues have a predominant aerobic metabolism, this process is of significant protective value.

In addition to the microsomal enzyme components, xanthine oxidase (*70,71*) is also capable of catalyzing the reduction of metronidazole and other nitroimidazoles. *N*-(2-hydroxyethyl)oxamic acid and acetamide form in this system when metronidazole is reduced (*70*). These metabolites are also formed by intestinal flora and can be detected in the urine when metronidazole is administered to to rats or humans (*72–74*). Together, *N*-(2-hydroxyethyl)oxamic acid and acetamide account for all the carbon and nitrogen atoms of metronidazole except for the nitrogen atom in the nitro group.

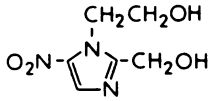
However, most of the metabolites of metronidazole excreted in urine contain the nitro group (*75–77*). The main metabolites are the sulfo and glucuronic conjugates and the oxidation products of metronidazole: 1-(2-hydroxyethyl-2-hydroxymethyl-5-nitroimidazole, 1-(2-hydroxyethyl)-2-carboxyl-5-nitroimidazole, and l-acetic acid-2-methyl-5-nitroimidazole.



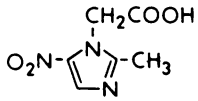
METRONIDAZOLE GLUCURONIDE CONJUGATE



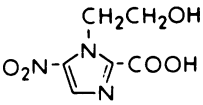
METRONIDAZOLE SULPHATE CONJUGATE



HYDROXYLATED DERIVATIVE



ACID DERIVATIVE



ACID DERIVATIVE

Nevertheless, the absence or low content of reductive metabolites *in vivo* does not imply that nitro reduction to the anion radical has not occurred. Whole animal studies which show no net formation of products may be misleading in ascertaining the importance of free radical intermediates, because futile metabolism is characteristic of many classes of free radicals (*26*).

The mechanism of the neurotoxicity of nitroimidazoles is not completely understood. Based on results of inhibition of plant fatty acid synthesis by metronidazole and several other nitroimidazoles, some authors (*78*) have proposed this as a possible mechanism of toxicity. However, no experiments with mammalian systems have been reported to date.

With regard to the mechanism of radio and chemosensitization, most authors have preferred mechanisms operating at the cellular level, including thiol depletion and inhibition of DNA damage repair (*79*). However, recent evidence has indicated that changes in pharmacokinetics, possibly through inhibition of drug-metabolizing enzymes in liver, may also be important for the chemosensitization (*80*).

Finally, recent studies with ronidazole (*81–86*) have shed light on the possible mechanism of toxicity of 5-nitroimidazoles to animal tissues. Under different experimental conditions leading to enzymatic reduction of this compound, reactive metabolites that bind covalently to protein were produced (*81–86*). This covalent binding was effectively prevented by reduced glutathione and cysteine. The principal targets of protein alkylation were cysteine thiols (*83*). A ronidazole–cysteine adduct could be isolated (*85*) in *in vitro* incubations of rat liver microsomes, suggesting that this adduct may account for the observed binding of ronidazole to microsomal protein and for the presence of intractable

drug residues in the tissues of the animals treated with this compound.

Genotoxic Action of Nitroimidazoles

Nitroimidazoles interact with DNA (*19,87–89*) and have mutagenic action in bacteria (*12,90*). Reports of the nature of the target site of nitroimidazoles in the DNA are conflicting. Some authors have shown that the interaction of chemically reduced nitroimidazoles with DNA is directly proportional to the G + C content (*61*). This was also confirmed by experiments in which guanine was modified by chemically (*91*) or electrolytically (*87*) reduced nitroimidazoles without affecting the DNA backbone. However, other investigators have shown fragmentation of the DNA (single- and double-strand breaks) by the activated nitroimidazoles and a preferential release of thymidine phosphate after electrolytic reduction of nitroimidazoles in the presence of DNA (*19,87,88,92*).

Metronidazole and other 5-nitroimidazoles are "direct mutagens" in *S. typhimurium* TA 100 (i.e., they induce mutations in this bacteria without the addition of exogenous "activating" systems such as rat liver S-9) (*93–99*). Addition of rat liver S-9, however, enhances the mutagenic activity of metronidazole, but only under anaerobic conditions (*96*). A survey of 11 nitroimidazoles showed that only those compounds that have a reducible nitro group are active (*93*). The analysis of the mutagenicity tests performed with different mutant strains of *S. typhimurium* (*94–96*) showed that error-prone repair processes are involved in their mutagenic action.

Mutagenicity of metronidazole and other 5-nitroimidazoles is probably the result of metabolic activation to reduced forms, rather than to the unmetabolized compounds *per se*. Experimental data from mutagenicity assays of different nitroimidazoles support this conclusion. Thus, nitroimidazoles are able to mutate *S. typhimurium* TA 100, which has both oxygen-insensitive and oxygen-sensitive nitroreductase activity, with or without the addition of rat liver S-9, under aerobic and anaerobic conditions (*96*). However, TA 100-FRI, a mutant of TA 100 lacking oxygen-insensitive nitroreductase, is not aerobically mutable by nitroimidazoles in the absence of the rat liver S-9 fraction (*96,97*). Since this strain of bacteria retains oxygen-sensitive nitroreductase, it is mutable anaerobically with or without the S-9 fraction of rat liver (*96*).

Mutagenic activity of nitroimidazoles has also been detected in *Klebsiella pneumoniae* (*12,90*), *Escherichia coli* (*98*), and yeast (*99*).

Recent work with several nitroimidazoles suggests that ring opening of partially reduced compounds may lead to biologically active agents. For example, metabolism of metronidazole by intestinal bacteria yields acetamide, a compound known to be carcinogenic (*71*). In addition, misonidazole, when reduced by xanthine oxidase and xanthine, breaks down to glyoxal, whose reactivity with proteins and nucleic acids is well known (*100*).

Finally, with regard to the carcinogenicity of nitroimidazoles, the reports are conflicting and are reviewed elsewhere (*1,19*).

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